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Short Communication

Near wall void growth leads to disintegration of colloidal bacterial streamer

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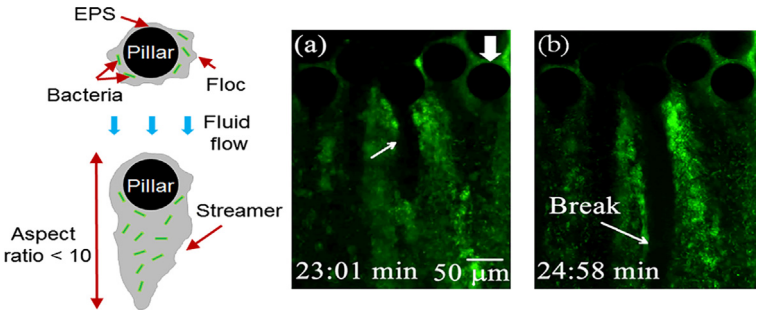
GRAPHICAL ABSTRACT



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ABSTRACT

We investigated the failure of thick bacterial floc-mediated streamers in a microfluidic device with micropillars. It was found that streamers could fail due to the growth of voids in the biomass that originate near the pillar walls. The quantification of void growth was made possible by the use of 200 nm fluorescent polystyrene beads. The beads get trapped in the extracellular matrix of the streamer biomass and acted as tracers. Void growth time-scales could be characterized into short-time scales and long time-scales and the crack/void propagation showed several instances of fracture-arrest ultimately leading to a catastrophic failure of the entire streamer structure. This mode of fracture stands in strong contrast to necking-type instability observed before in streamers.

1. Introduction

Bacterial streamers are filamentous biofilm-like structures that are usually known to form under sustained hydrodynamic flows [1]. Like biofilms, streamers consist of bacterial cells embedded in a matrix of self-secreted extracellular polymeric substances (EPS) and are excellent examples of soft materials of biological origin [1–4]. Streamers can also be considered extremely heterogeneous colloidal systems, as the bacteria are dispersed in an irregular fashion in the EPS. The basic colloidal nature of the system was critically underlined by the recent discovery of morphologically similar features that are found in particle laden polymeric flows [5]. Thus, bacterial streamers belong to the more generic category of 'colloidal streamer' formation [5] and they have been found to be relevant to a number of different applications.

Due to their morphology, streamers can colonize closed channels significantly faster than surface-hugging biofilms; recently streamers forming in very low Reynolds number conditions ($Re \lesssim 1$) have been implicated for their role in rapid fouling of

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biomedical devices [6–8], filtration units [9,10] and even colonization of porous media [1,11,12]. In many of these applications, a better understanding of deformation, fracture and failure of streamers is crucial [9]. Valiei et al. [12] had reported failure and disintegration of streamers in microfluidic device, but this phenomenon was not discussed in detail. Das and Kumar [13] had investigated instabilities and break-up of streamers when they were idealized as highly viscous liquid jets. In a later work, Biswas et al. [9] utilized a microfluidic device with micro-pillars to investigate far-from-wall failures of streamers. They focused exclusively on 'thin' streamers, i.e. streamers whose aspect ratio (η), i.e. ratio of longitudinal to transverse characteristic length, is typically >10, and found that these streamers could fail through a necking like failure mode in steady flow typical of ductile materials under creep. They also found a power law relationship between the critical strain (strain at instability) and fluid velocity scale, which yielded valuable insights into material behavior. Recently, Hassanpourfard et al. [14] showed that failure of streamers is not limited to the earlier stages but can also be found in the final clogged state of the device where localized failures lead to intricate water-channels coursing through the clogged biomass. Despite these studies, it is almost certain that more failure modes exist. This is because streamers like other biofilms represent a composite and extremely heterogeneous, active soft material. However, reporting and quantification of these phenomena is sparse.

One of the most fundamental challenges stems from the visualization issues since the EPS matrix embedding the microbe is very difficult to image due to its transparent nature. Furthermore, the complex nature of this biological system results in several overlapping sources of nonlinearity in addition to failure and instability. These include creep behavior of the polymeric EPS, fluid-structure interaction, moving interfaces and life processes, all of which cannot be independently controlled easily. In addition, time scale of streamer formation can vary considerably [7,11,12,15] and very long-time scales can let significant changes in the background conditions affecting these nonlinear behaviors.

In this communication, we report an entirely new type of streamer failure mechanism. This type of failure originates near the micropillar wall, rather than further downstream, as previously noted, and yet distinct from shear failure at the micropillar wall typically seen at higher flow rates [9]. We utilized a floc-mediated [9,11,14] rather than biofilm-mediated [7,12] streamer formation, where either of these refers to the mode of inception of the streamers [1]. Floc mediation allows for rapid streamer formation which helps us isolate mechanical factors and reduce streamer formation time thus reducing biophysical complications such as cell division [11]. A microfluidic device was specially fabricated to let the streamers to freely form from micropillars into the downstream flow without any further attachment to the surfaces on the free side. Our imaging showed that the inception of failure occurred with a pronounced void almost with the geometry of a small coin close to the point of attachment. This mode of failure occurred only after the streamer structure was already well formed (i.e. when streamer length is several times the pillar characteristic length). Once this 'crack' was observed, it was found to rapidly extend, with repeated instances of fracture-arrest, finally leading to the failure of the streamer biomass. While, the mechanisms behind crack-arrests are not fully known, we found this behavior repeatedly, always originating near the pillars and only for some but not all streamers. This failure mode did not occur anywhere farther down the streamer length. The flow rate was kept constant for individual experiments. To the best of our knowledge, this is the first report on this type of failure mode in streamers formed in microfluidic environments.

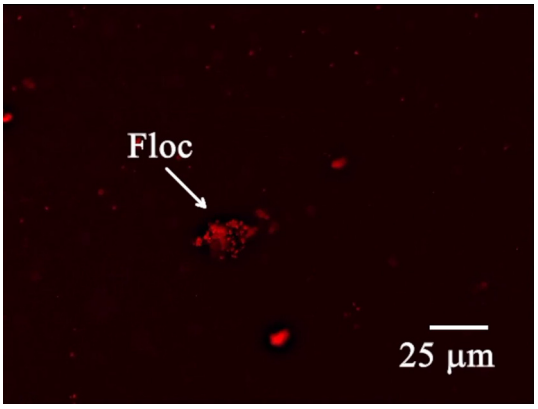
2. Materials and methods

2.1. Microfluidic chip fabrication

A PDMS (Polydimethylsiloxane) microfluidic device was made by using traditional photolithography technique for this study. A $\acute{4}$ silicon wafer was utilized to make the master mold of the microfluidic device. The microfluidic device design consists of a straight channel with a single inlet and outlet (Fig. 1a). The length of the channel, L, was 11.5 mm and its width, W, was 0.436 mm. In the central section of the channel, 14 micro-pillars were arranged in a staggered pattern. Using the photolithography process, as detailed by Hasanpourdfard et al. [16], PDMS micro-channels were prepared from a silicon master. The PDMS micropillars had a diameter, d, of 50 μm, height, h, of 50 μm and the pore-gap, p, of 10 μm (Fig. 1b). The dimensions w_1 and w_2 demarcated in Fig. 1b are 60 μm and 104 μm, respectively. Glass cover-slips (thickness 0.13–0.17 mm) (Fisher Scientific, ON, Canada) and PDMS were bonded by using oxygen plasma, followed by annealing at 70 °C for 10 min.

2.2. Bacteria culture preparation

We used *Pseudomonas fluorescens* CHA0 (wild-type) [17] bacteria strain for this study. This gram-negative aerobic bacteria is found naturally in water and soil and plays a vital role in plant health [18]. The bacteria were genetically modified to produce a green fluorescent protein (GFP) constitutively and hence appeared green under fluorescence imaging. The bacteria strain was taken from −80 °C collection and streaked on Luria Bertani (LB) agar plate in a zigzag pattern. The plate was incubated overnight at 30 °C. Subsequently, a single colony was taken from the plate and put into liquid LB broth and was incubated for 26 h in a shaking incubator (Fisher Scientific, ON, Canada) at 150 rpm and 30 °C. The longer incubation time was employed to generate bacterial flocs [11]. This bacteria culture was mixed with 200 nm red (Excitation/Emission – 580/610 nm) fluorescent amine-coated polystyrene (PS) microspheres (Thermo Fisher Scientific, MA, USA). The final average concentration of these tracer PS beads in the injected mixture into the microfluidic chip was 0.02% (v/v). Confocal microscopy analysis revealed that only a small fraction of the tracers was absorbed in the EPS matrix and the final volume fraction of tracer particle vis-à-vis cells was insignificant (Fig. S1). The Young's modulus of polystyrene particles and bacteria cells are ($\sim O(10^3$ MPa)) [19] and $\sim O(10^2$ MPa) [20], respectively. Thus, the overall impact of the tracer particles on the mechanical behavior of streamers can likely be neglected. This



Supplementary Video 1. This video shows the 5 min of ex-situ observation of floc and particles in the bacteria culture prior to the injection to the microfluidic device. The video shows that the Brownian motion of the tracer particles inside the floc is negligible as compared to its Brownian motion in the liquid. Video is not real time. It is 16× speeded up.